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## GREEN PLANTS AND THE REACTIONS OF IRON

NORMAN ASHWELL CLARK

In several of its aspects the problem of the availability of iron for plants is still unsolved. If the problem is not unique, it is at least unusual. In general, the plant takes its elements in simple combinations—almost, the simpler the better. Phosphorus is picked up as phosphate, nitrogen as nitrate or ammonia, sulphur as sulphate, and metals can be given as salts—nitrates, sulphates and chlorides. But try putting ferric chloride into a nutrient solution with a reaction around the neutral point! The plant turns yellow; the leaves become chlorotic, and there are all the indications of iron deficiency.

The need of the plant for iron was not appreciated until most of the other required elements were known to be essential for growth. Liebig does not give it in his list. Later, iron was recognized as essential and was thought to be present in chlorophyll, the green coloring matter of the leaf. Willstätter showed that magnesium is the metal in chlorophyll, but this compound is not formed unless iron is present.

In the early days of nutrient solutions many of the media used were found to produce chlorotic plants. The effect of the acidity of the solution on the availability of iron was not always appreciated, even when it was known that in the absence of organic matter increasing alkalinity tended to precipitate ferric hydroxide. This hydroxide precipitates far on the acid side of neutrality. Quartaroli (7), in Italy, showed that in a concentration of ferric chloride of 0.5 millimoles per liter, 95 per cent was hydrolyzed in about six hours. Hopkins and Wann (4) found only one one-hundredth of the iron from ferric sulphate in a salt solution of pH 7 after standing over night. Olsen (5), in Denmark, pointed out that even at pH 3.5 a large part of the iron precipitated from ferric chloride in a Knop's solution, while at pH 7 less than 0.001 milligrams per liter of ferric ion remained. He pointed out that chlorosis in plants under such conditions was to be expected.

Iron in carbon compounds is frequently available when inorganic iron is not—particularly in alkaline solutions. Certain organic compounds of iron—for example, the citrates—form complex iron ions which are not precipitated in neutral or alkaline solution,

or only break up very slowly. These enable the plant to obtain the necessary iron. Tartrates have been held to behave in a similar way, and Burk (2), at the Fixed Nitrogen Laboratory in Washington, has pointed out the possibility that the iron is available when absorbed or combined with humic acids.

There is no agreement as to the reason for the availability of the iron in organic combination. Comber, in England in 1922 (3), advanced the hypothesis of the direct utilization of colloidal iron; Olsen, in Denmark, suggested the absorption of complex iron combinations; Thomas, in Pennsylvania, (8) doubts both explanations and states that iron citrates and tartrates are colloidal, giving ferric ions, but with slow hydrolysis and final precipitation. Dr. C. L. Fly, working in our laboratories at Iowa State College, found that if iron citrate was added to salt solutions with increasing concentration of iron (16 to 80 mg. per liter) and the pH of the media also varied from the acid 4.8 to the alkaline 8.8, after growing the plant *Lemna* for 5 days in the solutions, in all cases the content of iron was close to 1 mg. per liter, the greater part precipitating as ferric hydroxide.

In inorganic combination iron hydrolizes at a pH of 5 and above very strongly, and the precipitated ferric hydroxide seems unable to supply the needed iron. We have grown *Lemna* in solutions containing precipitated ferric hydroxide with the roots touching the precipitate, but chlorosis resulted. If the plant can grow at a pH of less than 5, it can use iron as chloride or sulphate, for precipitation does not take place or is very slow; but the majority of plants prefer reactions of media nearer neutrality, pH 7, and here the ferric hydroxide is formed. On the other hand, most soils are well above pH 5 and yet cause no chlorosis; this may be due to combinations of iron with organic matter, similar to ferric tartrate or citrate, which prevent the precipitation and cause it to remain in some form which is available even at neutral or alkaline reactions.

This problem thus becomes involved with another — the question of auximones and auxins, and the influence of organic matter on the growth of plants. Small amounts of complex organic material, for example soil or manure, have been found to be exceedingly beneficial to plants in inorganic solutions. Bottomley (1), at the University of London, soon after the discovery of vitamins and the recognition of their essential nature in animal nutrition, developed the idea of a vitamin for green plants — just as essential for plants as the others for animals. He called these plant

accessory substances 'Auximones.' In our work with *Lemna*, this conception was shown to be incorrect. We were able to grow the *Lemna* on pure inorganic salts without traces of organic matter, and in the absence of all bacteria or other micro-organisms; but there remained the fact that organic material could influence the plant, both in rapidity of growth, reproduction and composition.

The recognition of this influence of organic matter goes back to the seventeen hundreds, when humus was supposed to be the food of plants. After the theory was disproved — around 1800 — and de Saussure and others had discovered the facts of photosynthesis, humus for many years was considered only as a conditioner of the soil — an indirect effect on the plant. The study of the nutrition of yeast, and the direct effect of organic matter — Bios — on its reproduction, the discovery of vitamins for animals, and Bottomley's suggestion of auximones for plants — all these focussed attention on the possibility of direct effects of organic matter on growth.

In 1932 Burk (2), at Washington, came to the conclusion that this direct effect was connected with iron. In his experiments with *Azotobacter*, the nitrogen fixing organism, he found that extracts of humates from soil stimulated growth markedly, and the amount of stimulation was directly proportional to the quantity of iron in the humus — the organic fraction itself was inactive. Synthetic humates did not stimulate unless the iron compounds had been formed. He therefore suggested that Bios in yeast nutrition, and other organic stimulators of growth in plants, functioned because of their capacity to form highly available iron combinations and not because of the organic substance itself. In view of the recent work on auxins, with special organic compounds, such as indole acetic acid, stimulating particular growth — for example, root production — this conclusion may need to be modified.

In our laboratory Dr. C. L. Fly obtained results with citrates similar to Burk's with humates, but on green plants. An increase in the content of iron as iron citrate increased the rate of reproduction of *Lemna*. At a pH of 4.7 iron citrate gave no better growth than the chloride, but with an increase in pH the superiority of the citrate was marked. There is both a minimum and a maximum content of iron as citrate for best reproduction. Dr. Fly found that if the iron concentration was 2 mg. per liter or less, the higher the pH the poorer the plants — at pH 7 the plants died. With higher concentrations of iron, reproduction was excellent up to pH 8.

Burk received confirmation from Olsen in Denmark (5). Olsen had worked with Lemna and had checked Bottomley's observations on auximones. He, also, came to the conclusion that the reason for the stimulation by the organic matter was the presence of complex iron combinations in the humus extract. It appeared, therefore, that organic matter might affect the plant directly in two ways — by the auxins, with their specific effects on cell division, elongation or root formation, and by the iron compounds which allow the plant to take up the iron at high pH values. There is a possibility however, that the problem may be yet more complex.

A few months ago Olsen published some further studies on iron (6). He pointed out that the leaves of fruit trees which were chlorotic had been shown to have as much or more iron in them as in green leaves, and that this indicated the problem was not one of availability in the solution, but of use after uptake. He checked the findings on chlorotic corn and other plants; the iron content and also the content of other elements, calcium, magnesium potassium and phosphorus, were always higher in the chlorotic leaf than in the green leaf.

Olsen claimed another unexpected fact — that contrary to earlier findings, plants *can* obtain iron from ferric chloride in alkaline solutions. For example, while Lemna grows poorly at pH 4 in a Knop's solution, is good around 5 and chlorotic at 6 and 7, at pH 8 the growth is again good, with no chlorosis. This, he states, held for corn and other plants, and any variation was in the direction of less chlorosis at pH 6 or 7 — for example, with barley. The curve showed two points of maximum growth, but by cutting down the phosphate, these two points merged and there was no chlorosis at any pH. His theory is that the iron is taken up from neutral or alkaline solutions, but under these conditions is precipitated in the vascular bundles as ferric phosphate and this stops the formation of organic matter. Ferric citrate does not give up the iron to the phosphate and Olsen believes the iron passes through the plant as citrate.

Some of these conclusions are not easy to accept. Mr. Sieling, who has been working with me on iron-humus combinations, has checked the growth of Lemna at low phosphate concentration. He finds no chlorosis at pH 7 or 8. With our usual solution, however, we do not obtain the two maxima with ferric chloride — the Lemna will not grow at a much greater pH than 5. We are continuing the work, but the problem, as I said at the beginning, in some of its aspects is as yet unsolved.

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